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## **KILLING OF INVERTEBRATA IN AN EXPANDED AND NATURAL CONDITION.**

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It would seem worthless for one to write upon this subject when so much has already been said and so many, I might almost say, hundreds of formulæ have been devised for the killing of the various invertebrates; but since I have had the pleasure of using nearly all of these different formulæ at the seashore, as well as inland, and have tested their respective values on hundreds of invertebrates, I trust that my experience may be of benefit to others and save them the expense, worry, and inconvenience I have passed through in gone but not forgotten days. There is no need of taking a trunk-full of chemicals to the seashore or to the mountains and lakes; neither are they essential in your laboratory. It must be remembered that I am speaking of the chemicals to be used for *killing* only, and not of those for preserving, mounting, etc.

Lay aside your scores of formulæ—a very few indeed are necessary in extreme cases, but these are so few that they apply only to the specialist in that particular case.

All the chemicals that are needed to kill invertebrates fully expanded are the following:

*Osmic Acid*.—This comes in glass tubes in the form of crystals, one gramme in a tube. It is to be used as a 2 per cent. aqueous solution. If a 1 per cent. solution is to be used it can be readily made, as needed, in a small vial. Osmic acid is to be used to kill all *protozoa*, *hydra*, many *minute worms* (naididæ, etc.), rotifera, *certain hydroids*, *small jelly-fish*, *small ctenophora*, and many kinds of minute animals and embryos. For protozoa a 1 per cent. solution is to be used, the animals placed in as small an amount of water as will admit of their proper expansion, and as soon as fully expanded a drop of the 1 per cent. solution is to be dropped upon them, when they will be killed before they can contract. A 1 per cent. solution may answer for rotifera, small jelly-fish, small ctenophora, etc., if

placed in a small amount of water in a watch-glass; but for the larger, and especially for the hydroid polyps, a small branch of which is to be placed in a watch-glass of water, it will be necessary to use a 2 per cent. solution, since there is more water and the animals are larger and harder to kill in this case. In short, osmic acid is to be used for very minute animals.

*Mercuric Chloride*.—A saturated solution in 50 per cent. alcohol, to be used hot. This will be found useful for many *worms*, *turbellaria*, etc.—*some mollusks* and other small animals not readily killed by the other methods.

*Erlicki's Fluid*.—This fluid is composed of cupric sulphate, 1 part; bichromate of potassium, 5 parts; water, 200 parts. If traveling, it will be found convenient to have it put up in packages of the proper proportions, so that each package will require one quart of water. It can then be made at any time and place, as needed. This fluid is used as a hardening agent for histological purposes, but it is also the best fluid for killing *jelly-fishes*, large *ctenophora*, *siphonophora*, and allied animals. The animals are to be placed alive into a large quantity of this fluid, and are to remain in it for three days, when they can be placed in increasing strengths of alcohol.

*Alcohol*.—It is this simple alcohol to which I wish to call especial attention, and of which I claim the greatest good can be made. Aside from its preservative qualities, I find it to be more useful for killing *all kinds of animals* above protozoa than all the other chemicals and formulæ that have yet been devised added together. I have not tried to use alcohol as a killing agent for the protozoa, since osmic acid does all that could be asked, and from the nature of the way in which alcohol is to be used it would be very tedious. It is a rule, I think, without an exception, that every species of invertebrata above protozoa that is liable to contract or change its form when disturbed or killed can be killed by the use of alcohol so as to remain in an expanded and natural position. The whole secret is to kill the animal slowly and unconsciously—to use such a strength of alcohol as will kill in time, but such as will not disturb or irritate it in the least. The animals expand, gradually become insensible, and die.

The strength or per cent. of alcohol to be used varies with different species, so that no rule can be given. Some animals will allow of a wide variation of strength and yet be killed expanded, while other species, such as, for instance, *crystatella* (a polyzoan), are very sensitive and will not allow of a variation of 1 per cent. The method

to be used is to partly fill a bottle or a dish with pure water, and to add to this enough alcohol to produce the required strength, shake or stir the liquid thoroughly, and when quiet quickly transfer the animals from their natural element to the weak alcohol and allow them to remain there undisturbed. If the alcohol be too strong they will not expand fully, and if too weak they will not be killed when they have expanded. Experience is the only guide, and one will soon become able to gauge the strength for different animals. It is well to have several individuals, if possible, in order to ascertain the best strength of alcohol to be used. If the animal starts to expand and suddenly contracts when not disturbed mechanically, it is good evidence that the alcohol is too strong and has irritated it. It will be found better to thoroughly mix the alcohol and water before placing the animals in it, since, if the animals be placed in the liquid and alcohol then be added to produce the required strength, the mixing of the alcohol with the water will cause sudden and violent currents in the liquid, which will disturb the animals, and may cause stronger alcohol to flow over the animal and kill it or prevent its expanding.

If our animals live in salt or sea water it will be found necessary in most cases to use sea water, although in some cases fresh water is preferable. As a rule, however, use sea water if the animals live in the sea, and fresh water if they live in fresh water. Sea animals are also, as a rule, less sensitive to slight variations in the strength of the alcohol than are fresh-water animals. All kinds of hydroid polyps can be readily killed fully expanded and in any quantity by simply placing any number of branches in a vessel of from 8 per cent. to 12 per cent. alcohol in sea water. Of course, the vessel must not be disturbed by even slight jars, as it will prevent the zooids from expanding fully. They will be found dead in ten or fifteen minutes, and can then be placed in very gradually increasing strengths of alcohol, and will look as perfect as when living. It is well to ascertain when the zooids are dead by touching a few with a needle, so as to not disturb the colony. I have killed large colonies of various kinds of polyps in this manner, leaving each zooid with its tentacles fully expanded. Salt-water polyzoa, barnacles, sea anemones, sea cucumbers, coral polyps, worms, and, in short, everything, can be readily killed in a natural expanded condition in this way. By this method one can kill in a short time any number and any quantity of animals and have them in a respectable shape for study, classes, and the museum. Since we have to have alcohol

for preserving our specimens, it does not add to our equipment, and we have in it all that is really necessary for complete success. If our animals are to be used for histological study, they can be placed in hardening re-agents, after being properly killed by the above method.

The most difficult animals to kill in a natural and expanded condition are, I think, some of our fresh-water polyzoa, as *crystatella*. I have killed several colonies of *crystatella*, however, and have them where every animal has its lophophore and tentacles as fully expanded as in life, the entire colony differing in no way as regards position and extension from a fully expanded living colony. The proper way to do in such a case as this is to have several colonies, or to cut small pieces off of a colony and experiment with these until the proper strength of alcohol is determined, and then to transfer the colonies quickly from the fresh water into the alcohol water. They must then be left to themselves in a place free from jar, as even the walking across the room may cause the animals to contract or not to expand fully. They will expand in a few minutes and will die in the course of about twenty minutes. Great care must be taken not to disturb them when fully expanded and before they are dead, since if they become disturbed and contract at this time they will probably be unable to expand again. These animals are very sensitive, and it will take a little experimenting to obtain the proper strength of alcohol; if too weak it will not kill them, and if too strong they will not expand fully. I think about a 3 per cent. solution is a good one to work from.

A little experience is all that is needed to convince any one that this alcohol method is the best known means of killing all kinds of invertebrates above protozoa.

With this method at hand, there is no excuse for the miserable and unlikelike specimens of certain invertebrates with which our museums, laboratories, and cabinets abound, to say nothing of the class-room.